FURTHER OBSERVATIONS ON THE EFFECTS OF ROENTGENIZATION AND SPLENECTOMY ON ANTIBODY-PRODUCTION *

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In previous papers [1] I have recorded observations which show that roentgenization of white rats, dogs and rabbits at about the same time as antigen is introduced may restrain greatly the production of antibodies as measured by the antibody content of the serum. I have also noted [2] that in dogs splenectomy just before the injection of foreign blood was followed by a lower but otherwise typical antibody curve than is usually the case in dogs under otherwise comparable conditions. In the meantime, additional observations have been made on roentgenization and splenectomy under more diversified conditions, the results of which seem to merit a brief report.

SPLENECTOMY

Experiments on white rats gave results similar to those in dogs. Without exception the amount of lysin for sheep corpuscles was much less in the rats in which the spleen was removed at the same time that the blood was injected. As seen on chart 1, the lysin curves present the same general outlines, but in the splenectomized series the latent period is longer, the height and duration less than in the controls.

Rats weighing from 70 to 80 gm. were used; 1 c c of a 10% suspension of sheep blood per kilo of weight was injected intramuscularly immediately after the splenectomy. The curves (chart 1) are composite curves based on the titers of two rats killed on each day indicated, it being practically impossible to bleed the same rat many times. The control curve gives the titer of rats of the same age and size, treated in the same way, but not splenectomized. The titer gives the highest dilution of the serum that caused distinct lysis in a mixture of 0.6 c c consisting of 0.2 c c of a 5% suspension of sheep corpuscles, well washed, 0.0125 c c of guinea-pig serum, and the indicated amount of heated rat serum, the rest being salt solution. The tubes were incubated for two hours and then placed in the icebox until the next morning.

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[1] Jour. Infect. Dis., 1915, 17, p. 415; 1918, 22, p. 28.

[2] Ibid., 1909, 6, p. 78.

In rabbits splenectomy from one to six days before the intraperitoneal injection of 25 c c of sheep blood as a rule did not interfere with the production of lysin and precipitin; exceptionally, however, splenectomy shortly before the injection seemed to suspend completely the advent of antibodies into the blood.

Of 6 rabbits splenectomized 5-9 weeks before the injection, all save one developed about the usual amount of lysin and also precipitin in fairly high degree, though with a somewhat prolonged latency. Of 10 rabbits, all young and healthy, splenectomized from 5-9 weeks before the intraperitoneal injection of 30 c c of human

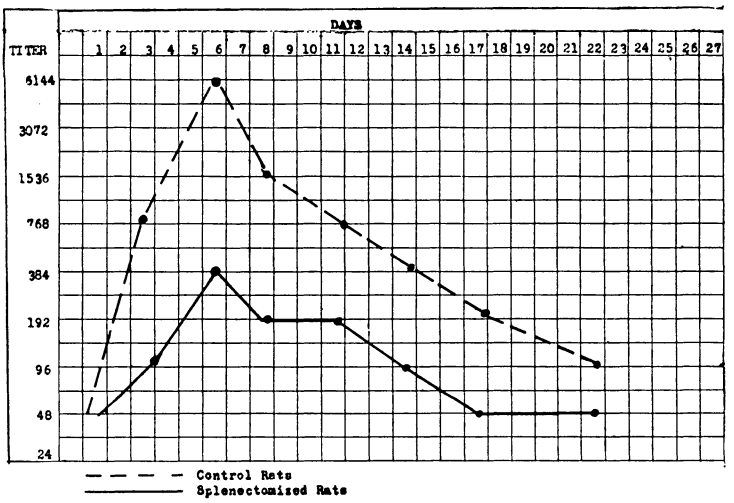

Chart I. Lysin in Normal and Splenectomized Rats.

blood, all but one failed to develop more than a trace of precipitin, but in all agglutinin of considerable strength developed as well as smaller amounts of lysin. The last result is of interest because it suggests that under certain conditions splenectomy, even some time before immunization, may restrain the formation of one kind of antibody more than others.

In the experiments 25 c c of sheep or human blood were injected intraperitoneally in one dose. The highest active dilution of the serum was determined in the case of the lysin and agglutinin tests, and the highest dilution of sheep or human blood in which the rabbit serum caused precipitate by the ring or contact method after 2 hours at the room temperature was determined in case of the precipitin tests. The lysin and agglutinin tests were carried out in mixtures of 0.6 c c containing 0.2 c c of a 5% suspension of washed corpuscles, heated rabbit serum, and in the lysin tests guinea-pig serum (complement), the

rest being salt solution. The complement dose was 0.006 c c in the tests for lysin or sheep corpuscles, and 0.02 c c in the tests for lysin for human corpuscles. All lysin and agglutinin mixtures were incubated for 2 hours and then placed in the icebox until the next morning.

Taken as a whole, my results correspond well enough with those of earlier observers, some of whom obtained inhibition of antibody-production from splenectomy (London,[3] Deutsch [4]), while others failed (Jakuschewitsch,[5] Kraus and Schiffman,[6] McGowan [7]), but minute comparisons are not worth while because of great differences in the experiments, e. g., mode of injection of antigen, measurements of antibodies, animals used, etc.

SPLENECTOMY AND ROENTGENIZATION

Table 1 gives details of an experiment on young dogs of the same litter in which roentgenization and splenectomy, alone and combined, greatly reduced the output of lysin after injection of goat blood. The small number of dogs represented precludes any conclusion as to which procedure may be most effective, but the results of splenectomy alone or combined with roentgen ray shortly before the antigen was injected seem the more striking.

In this, as well as in the other experiments discussed, the roentgenization was done in the Presbyterian Hospital by Earl Ball. The Coolidge tube was used, the focal distance was 8 inches, the current 5 to 6 milliamperes, spark-gap 8 inches. In the tables the dose is expressed in calculated Kienbach units. Usually two exposures were given, a major and one one-fourth as long the next day.

ROENTGENIZATION AND SPLENECTOMY AT HEIGHT OF ANTIBODY-PRODUCTION

Tables 2 and 3 and chart 2 give the results of new experiments [8] on the effect of the roentgen ray and splenectomy at or near the high point of the accumulation of antibody in the blood. These results indicate that neither roentgenization, as practiced, alone or combined with splenectomy, nor splenectomy alone or combined with roentgenization had any appreciable influence on the course and amount of antibodies in the blood when applied several days after the introduction of the antigen. The experiments covered by tables 2 and 3 concern in each case young dogs of one litter.

[3] Arch. d. Sc. biol., 1901, 8, p. 328.
[4] Ann. de l'Inst. Pasteur, 1899, 13, p. 688.
[5] Ztschr. f. Hyg. u. Infektionskr., 1904, 47, p. 407.
[6] Ann. de l'Inst. Pasteur, 1906, 20, p. 225.
[7] Jour. Path. and Bacteriol., 1911, 15, p. 262.
[8] Hektoen: Jour. Infect. Dis., 1918, 22, p. 28.

TABLE 1

ROENTGENIZATION (45 KIENBACH UNITS) AND SPLENECTOMY, SINGLY AND COMBINED, SHORTLY BEFORE AND 5 DAYS AFTER INJECTION OF GOAT BLOOD IN DOGS

Number of Days After Injection of Goat Blood	1 Roentgen Ray 2 Days Before Injection	2 Roentgen Ray 2 Days Before Injection and Splenectomy 5 Days After	3 and 4 Roentgen Ray 2 Days and Splenectomy 1 Day Before Injection		5 Splenectomy 1 Day Before Injection	6 Splenectomy 1 Day Before and Roentgen Ray 5 Days After Injection	7 Control
2	0	0	0	0	0	0	48
3	0	0	0	0	48	48	192
4	0	0	0	0	96	48	384
5	96	96	48	0	0	192	768
6	384	96	48	0	0	384	1,536
7	384	192	48	48	96	384	3,072
8	768	384	96	48	0	384	3,072
9	768	384	96	48	0	384	3.072
10							
11	192	96	48	48	192	192	1,536
12	192	96	48	48	0	192	768
13	192	96	48	48	0	192	384
14							
15	96	0	0	0	0	48	192
16							
17	96	0	0	0	0	96	192
18	96	0	0	0	0	48	192

TABLE 2

ROENTGEN RAY (45 KIENBACH UNITS) AND SPLENECTOMY, SINGLY OR COMBINED, IN DOGS AT OR NEAR HEIGHT OF PRODUCTION OF LYSIN FOR GOAT CORPUSCLES

Number of Days After Injection of Goat Blood	1 Roentgen Ray on 6th Day	2 Splenectomy on 6th Day	3 Splenectomy on 6th Day and Roentgen Ray on 7th Day		4 Control
3					
4	192	192	.....	.....	96
5	768	768	192	768	384
6	768	768	384	1,536	768
7	1,536	1,536	768	3,072	768
8	3,072	1,536	1,152	1,536	1,536
9	3,072	3,072	1,152	1,536	1,536
10	6,144	3,072	1,536	1,536	1,536
11	3,072	3.072	1,536	1,536	1,536
12	3,072	3,072	768	1,536	3,072
13	1,536	768	768	768	768
14	1,536	768	768	768	768
15	1,536	.....	384	768	
16	1,536	768	384	768	768
17	.....	.....	384	768	
18	1,536	768	.....	.....	768
19	.....	.....	192	384	
20	768	384	.....	.....	384
21	.....	.....	192	192	
22					
23	.....	.....	192	192	384
24	768	384			
25					
26	.....	.....	192	192	192
27					
28	768	192	.....	.....	192

TABLE 3

ROENTGEN RAY (45 KIENBACH UNITS) AND SPLENECTOMY, SINGLY OR COMBINED, IN DOGS AT OR NEAR HEIGHT OF PRODUCTION OF AGGLUTININ FOR RAT CORPUSCLES

Number of Days After Injection of Goat Blood	1 Roentgen Ray on 6th Day	2 Splenectomy on 6th Day	3 and 4 Splenectomy on 6th Day and Roentgen Ray on 7th Day		5 Control
4	96	96	...	...	96
5	192	192	0	0	192
6	192	192	192	96	192
7	384	384	192	192	384
8	768	768	384	384	768
9	768	768	768	384	768
10	768	768	768	576	768
11	384	768	...	...	384
12	384	384	768	384	384
13	384	768	384	384	384
14	192	384	384	192	384
15	...	...	384	192	
16	384	384	...	...	192
17	...	...	384	192	
18	384	384	...	...	192
19	...	...	384	192	
20	192	384	...	...	192
21	...	...	384	96	
22					
23	...	...	192	96	
24	192	384	...	...	192
25					
26	...	...	96	96	
27					
28	192	384	...	...	192

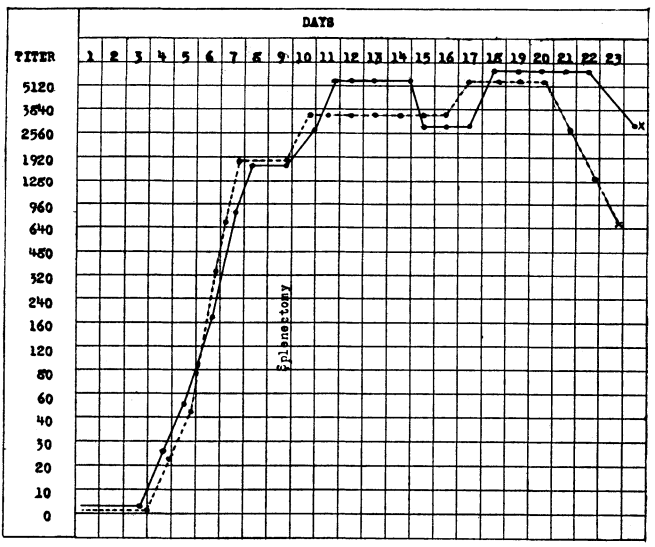

Chart II. Splenectomy at Height of Antibody Curve.

Beginning soon after splenectomy the red corpuscles were found more resistant to hypotonic solution than the corpuscles of nonsplenectomized animals. The increase in resistance seemed to be about the same in the splenectomized animals treated with roentgen ray as in those that were not; there was no change from the normal in the resistance of the corpuscles of animals subjected to the ray only. In the rabbits, too, splenectomy as a rule results in an increase.

Chart 2 illustrates the results of a study on 6 healthy young rabbits, each injected with 25 c c sheep blood, and in two of which splenectomy was made 9 and 10 days later, but without any effect whatever on the precipitin titer as compared with that in the controls.

These results are in full harmony with the results obtained by London [3] in an experiment on the effect of splenectomy some days after the production of hemolysin had started. I have reported previously that roentgenization of dogs when antibody-production is well under way has little or no effect on the antibodies in the blood.[8]

ROENTGENIZATION SIXTEEN DAYS BEFORE THE ANTIGEN IS INTRODUCED

Five young, healthy dogs of the same litter were given each an intravenous injection of rat blood; 16 days before 3 of the dogs had been roentgenized for 15 minutes and again for 3 minutes the day after (54 K. units). Two days before the injection of the rat blood the leukocytes ranged from 14,666 to 17,000 in the roentgenized dogs and in the two control dogs the counts were 11,333 and 15,666; the differential counts were normal. Table 4 shows that the agglutinin titer ran uniformly higher in the dogs treated with the roentgen ray than in the controls.

In another experiment 3 dogs were roentgenized for 20 minutes (60 K. units) and 15 days later injected with goat blood. These animals developed profound effects and died a few days after the injection without having produced hardly any lysin.

The first experiment indicates that under certain conditions the roentgen ray induces such changes in the body that the power to elaborate antibodies is increased. It lies near at hand to associate this increased power with regenerative changes in the lymphatic tissues and spleen after roentgenization.

In all the experiments on dogs, the antigen, 1 c c of 10% suspension of goat blood or rat blood per kilo of weight of dog, was injected intravenously. Only one injection was given. The figures in the tables give the highest active

dilutions of the dog serums in mixtures of 0.6 c c containing 0.2 c c of a 5% suspension of washed goat or rat corpuscles, the proper amount of dog serum, 0.0125 c c guinea-pig serum in the lysin tests, the rest being salt solution. The mixtures were incubated for 2 hours and kept in the icebox until the next morning.

TABLE 4

AGGLUTININ PRODUCTION IN DOG PREVIOUSLY ROENTGENIZED (54 KIENBACH UNITS)

Number of Days After Injection of Rat Blood	Roentgenization 16 Days Before Injection of Rat Blood and Again for 3 Minutes the Next Day			Control	
	1	2	3	1	2
3	48	24	24	96	48
4	384	192	384	96	192
5	1,536	192	384	384	192
6	6,144	768	1,536	768	192
7	6,144	768	3.072	1,536	384
8	3,072	1,536	6.144	1,536	384
9	6.144	3.072	3.072	1,536	768
10	3,072	3,076	1,536	1,536	1,536
11	3,072	3,076	3,072	768	1,536
12	1,536	1,536	3,072	768	386
13	1,768	1,536	1,536	768	384
14	354	768	384	384	192
16	192	384	192	192	192
18	192	384	192	192	192
22	192	384	96	192	48
25	96	192	45	96	96

SUMMARY

The results recorded show that splenectomy may diminish the output of antibodies especially when practiced about the same time the antigen is injected. In the rabbit, however, splenectomy under certain conditions may have little or no effect on antibody-production, as after a single large dose of sheep blood. On the other hand, even when made several weeks before injection of human blood, removal of the spleen seemed to interfere with the formation of precipitin, but further observations are needed to determine whether such selective effect occurs regularly under these circumstances.

On the whole, the effects of splenectomy at or near the time of injection of antigen appear variable and uncertain, more so perhaps than might be expected from the demonstrations that antibodies appear earlier in the spleen than in the blood, that antigen is fixed by the spleen,[10] and that in the presence of antigenic substances cultures of splenic tissue outside the body may produce antibodies.[11] And yet

[9] Pfeiffer and Marx: Ztschr. f. Hyg. u. Infektionskranksr., 1898, 37, p. 272; Cantacuzene: Ann. de l'Inst. Pasteur, 1902, 16, p. 552; Tsurumi and Koda: Ztschr. f. Immunitätsf., O., 1913, 19, p. 519.

[10] Leuckart and Becht: Trans. Chicago Path. Soc., 1911, 8, p. 202.

[11] Carrell and Ingebrigtsen: Jour. Exper. Med., 1912, 15, p. 287.

variations in results are really not surprising if we consider, first, the close relation of the spleen to the lymphatic tissues and the marrow, which are believed also to take part in the elaboration of antibodies and consequently may be capable of compensatory activities, to say nothing of the possibilities of accessory spleens; and, secondly, that the experiments of different investigators were made under diverse conditions in such important respects as kind, quantity, and mode of injection of antigen, measurement of antibody, etc. Perhaps the effects of splenectomy would not be so variable in larger series of experiments with particular effort to secure as high a degree of constancy of the controllable factors as possible.

The results of several experiments indicate clearly that after antibody-production is well under way, splenectomy has little or no effect on the course of the antibodies in the blood. I have noted elsewhere [8] that the usual effects of the roentgen ray and of benzene appear to be withstood when antibody-production is well started. We now find that splenectomy, even when reenforced with roentgenization, seems to be subject to a similar resistance; at any rate, the antibody-content of the blood was not diminished markedly by splenectomy and roentgenization at or near the height of the curve. The nature of this so-called resistance remains obscure.

It may be pointed out again that as time passes after roentgenization the power to produce antibodies may be increased, and it is suggested that this increase may be due to regenerative changes in the spleen and lymph nodes. We consequently must distinguish between the immediate and the later effects of the roentgen ray. That the ray may reduce antibody-production seemed a good explanation of the increased susceptibility of guinea-pigs to tuberculosis described by Morton.[12] Kellert,[13] however, could not confirm Morton's claim; he found that roentgenization rather increased the resistance to the tubercle bacillus at the same time as the guinea-pigs seemed to become more susceptible to secondary and contaminating infections. Corper [14] also failed to produce any distinct effect on the gross tuberculous lesions in guinea-pigs by a single exposure to the roentgen ray. These contradictory results invite further experiments, not only on the effect of roentgenization on antibody-production, but also on phagocytosis and other cellular activities.

[12] Jour. Exper. Med., 1916, 24, p. 419.
[13] Jour. Med. Research, 1918, 39, p. 93.
[14] Am. Rev. of Tuberculosis, 1918, 2, p. 587.